In the Specification

Applicant presents replacement paragraphs below indicating the changes with insertions indicated by underlining and deletions indicated by strikeouts and/or double bracketing.

Please add the new paragraph at page 1, line 5, immediately following the title as follows:

Related Applications

This application is a continuation of US Serial No. 09/355,254 filed February 22, 2000 and now pending which claims priority to PCT Patent Application No. PCT/EP98/00367, filed January 23, 1998, and published in English as WO 98/32462, which in turn claims priority to EP Patent Application No. EP 97101019.4, filed January 23, 1997

Please replace paragraph 4 beginning at page 6, line 24 with the amended paragraph/line as follows:

The expression of individual genes is a rather complex process. These processes are mediated by several specific regulatory DNA regions found in the promotor regions of almost all genes. These regulatory sequences are frequently referred to as response elements. They are binding sites for sequence-specific DNA binding proteins which are called transcription factors. Some transcription factors are general purpose factors (basal transcription factors) required for transcription of all genes while others act on specific genes or classes of genes by binding in a sequence-specific manner to response elements and other sequence ~~motives~~ motifs within the corresponding gene promotors. The expression of many of these transcription factors is developmentally and also tissue-specifically controlled and is itself subject to the action of other transcription factors and other accessory proteins such as nuclear receptors. Binding sites for transcription factors are often clustered and a variety of transcription factors have been found to form complexes with others or to compete with others for binding to overlapping DNA-binding ~~motives~~ motifs. Several structural ~~motives~~ motifs have been found within those regions of transcription factor proteins recognizing and contacting DNA. Within each of these structural ~~motives~~ motifs there are often families of related proteins that recognize similar DNA sequences and are conserved throughout the eukaryotic kingdom.

Please replace paragraph 3 beginning at page 11, line 11 with the amended paragraph/line as follows:

Those preferred sequences of the present invention differ slightly but biologically significantly from the CpG ~~motives~~ motifs as disclosed in the prior art.

Please replace paragraph 7 beginning at page 11, line 23 with the amended paragraph/line as follows:

As regards the part of said binding site for said transcription factor or its complementary sequence, it is preferred that said part is a motif or a complementary sequence thereof. ~~Motives~~ Motifs of transcription factors are well known in the art and need not be discussed here any further.

Please replace paragraph 3 beginning at page 12, line 16 with the amended paragraph/line as follows:

It is possible to use in accordance with the present invention a wide variety of antigens. Preferred antigens are selected from the group comprising peptides, polypeptides, ~~steroides~~ steroids and tumor cells.

Please replace paragraph 2 beginning at page 16, line 7 with the amended paragraph/line as follows:

The pharmaceutical composition of the invention may advantageously be tested in mice. In such experiments mice were usually immunized by way of the hind footpad with 50 µl per foot of peptide liposome preparation. After four days, the draining popliteal lymph nodes (LN) were removed and a single-cell suspension was prepared. The cells were cultured for four days in the presence of IL-2 and a chromium release assay was performed utilizing the ~~syngenic~~ syngeneic target cell EL-4 or the cell line EG-7 which is transfected with the gene for ovalbumin and thus presents ovalbumin peptides as antigen (Fig. 1). In some experiments EL-4 pulsed with the MHC class I (K^b[b]) restricted ovalbumin peptide SIINFEKL (SEQ ID NO: 3) was used as the target for kill.

Please replace paragraph 7 beginning at page 16, line 28 with the amended paragraph/line as follows:

(c) repeating steps (a) and (b) one ~~ore~~ or more times until a non-toxic nucleic acid molecule has been identified.

Please replace paragraph 2 beginning at page 17, line 5 with the amended paragraph/line as follows:

An oligonucleotide that has been identified to be beneficial in accordance with the invention is IL-12p40 AGCTATGACGTTCCAAGG (SEQ ID NO: 10).

Please replace paragraph 6 beginning at page 18, line 21 with the amended paragraph/line as follows:

Analysis of a relevant cell surface marker after treatment with ssDNA. The IL-2 receptor binds and transduces a proliferation signal from IL-2 to cells of the immune system. The TRE sequences vary in their ~~simulatory~~ stimulatory capacity for inducing IL-2 receptor expression. Some TRE are inhibitory indicating a potential use for negative immunmodulation.

Please replace paragraph 7 beginning at page 18, line 26 with the amended paragraph/line as follows:

Figure 6: Tumor regression and control with ssDNA. ssDNA induces regression of ~~prexistant~~ preexistent tumor.

Please replace paragraph 7 beginning at page 19, line 21 with the amended paragraph/line as follows:

Three sequences containing the sequence motif of 5'Pu-Pu-CpG-Py-Py-3' are described in the literature for having immunostimulating properties. One sequence is derived from the ampicillin resistance gene of E. coli, here termed AMP (TCATTGGAAAACGTTCTTCGGGGC; SEQ ID NO: 1). The second sequence is derived from a BCG gene and is termed BCG-A4A (ACCGATGACGTCGCCGGTGACGGCACCACG; SEQ ID NO: 2). The third is a synthetic sequence claimed to be a prototype of bacterial CpG sequences, referred to by Krieg et[.] al. as 1668 (TCCATGACGTTCCTGATGCT; SEQ ID NO:

4). These sequences were synthesized to include a phosphorothioate linkage to reduce destruction by DNase. These oligomers served as an adjuvant in combination with ovalbumin to induce a cytolytic T cell response.

Please replace Example 3 spanning pages 21-22 with the amended paragraphs as follows:

Due to toxicity, the need is established for the discovery of non-toxic sequences for safe human and animal use. Since toxicity is at issue when developing vaccine adjuvants and therapeutics, we were interested to develop oligomers that circumvented toxicity but retained immunostimulatory properties. We screened eukaryotic sequences displaying the absence of lethality but maintaining immunostimulatory qualities. One such sequence was the cyclic AMP response element (CRE) which is the consensus binding site for the transcription factors CREB/ATF as well as the AP-1 family, sequence (GATTGCCTGACGTCAGAGAG; SEQ ID NO: 8) [Roesler, W. J. et al., J. Biol. Chem. 263, 9063-9066 (1988)]. Table 2 demonstrates the loss of lethality of the CRE sequence. To further evaluate the sequence specificity of these effects we made sequence exchanges between CRE and 1668. An exchange of only two nucleotides between CRE and 1668 resulted in a loss of lethality (Table 2).

Table 2 Sequences of oligomers and death due to lethal shock

a		
1668	TCCAT**GACGTT**CCTGATGCT	(SEQ ID NO: 4)
CRE	ATTGCCT**GACGTC**AGAGAGC	(SEQ ID NO: 5)
1668-CA	TCCAT**GACGT*CA***CTGATGCT	(SEQ ID NO: 6)
CRE-TC	*ATTGCC*T**GACGT*TC***GAGAGC	(SEQ ID NO: 7)
b		
1668	5/5	
CRE	0/5	
1668-CA	0/3	
CRE-TC	3/3	

Lethality was determin[in]ed as in [e]Example 2. The 1668 sequence fortuitously contains a combination of transcription response elements, namely the transcription factor binding sites (TGACGTTCC). This element represents the binding site for HSVIP04 (ATF), HSINS04 (CREB half site), CAMV35SR03 (HBP-1a yeast) or ADE422 (AP-1) in combination with an HSIL606 site which is a repressor site (sequence analysis from EMBL database Heidelberg). This sequence can be found in the 5' non-coding regions (promoters) of several eukaryotic cytokine genes including human IL-13 promoter and [Il]IL-12 p40 intron 1. The CRE sequence contains all the response elements cited above except for HSIL606 and it contains the full CRE pa[l]lindromic sequence (TGACGTCA). In accordance[s] with the invention, the CRE sequence did not induce death and changes in the 1668 eliminate toxicity.

Please replace paragraph 8 beginning at page 23, line 26 with the amended paragraph/line as follows:

We have described the use of liposomes in combination with Quil A or QS-21 to induce cytolytic T cells (CTL) to either soluble antigen or peptides [Lipford, G. B., Wagner, H. & Heeg, K., Vaccine 12, 73-80 (1994), Lipford, G. B. et al., J. Immunol. 150, 1212-1222 (1993)]. Liposome entrapped antigen alone was an ineffective inducer of CTL activity, but with the addition of immunostimulatory saponins the inoculum became effective. To test the in vivo T cell immunomodulatory potential of oligomers we utilized this vehicle to demonstrate primary activation of CTL. Figure 1 shows a substantial primary CTL response induced by an inoculum of ovalbumin liposomes plus ssDNA matching transcription response elements. The lytic units value interpolated from these curves was approximately 500 L.U. as compared to <20 L.U. for ovalbumin liposomes only (Table 3). CTL memory, an important quality for vaccine protection, could also be demonstrated with these inocula. If mice were rested for two weeks after the first injection and reinjected with the same inoculum, CRE recalled CTL displaying lytic units measured at approximately 1500 L.U. (Table 3). Additional, when the inoculum was formulated with the immunodominant K^b restricted ovalbumin peptide SIINFEKL (SEQ ID NO: 3), the oligomers induced a specific primary CTL response. Thus, oligomers serve as a strong in vivo stimulus resulting in T cell activation and the proliferation of antigen specific CTL effectors. The inoculum can contain protein or peptide as the target antigen.

Please replace Table 4 beginning at page 25, line 1 with the amended paragraph/line as follows:

Table 4 Sequences of eukaryotic TRE tested

CRE	GATTGCCTGACGTCAGAGAG	(SEQ ID NO: 8)
IL-13	GGAATGACGTTCCCTGTG	(SEQ ID NO: 9)
AP-1	GCTTGATGACTCAGCCGGAA	(SEQ ID NO: 11)
SP1	TCGATCGGGGCGGGGCGAGC	(SEQ ID NO: 12)
C/EBP	TGCAGATTGCGCAATCTGCA	(SEQ ID NO: 13)
E[G]RG	AGCGGGGGCGAGCGGGGGCG	(SEQ ID NO: 14)
GAS/ISRE	TACTTTCAGTTTCATATTACTCTA	(SEQ ID NO: 15)
SIE	GTCCATTTCCCGTAAATCTT	(SEQ ID NO: 16)
STAT1	TATGCATATTCCTGTAAGTG	(SEQ ID NO: 17)
STAT3	GATCCTTCTGGGAATTCCTA	(SEQ ID NO: 18)
STAT4	CTGATTTCCCCGAAATGATG	(SEQ ID NO: 19)
STAT5	AGATTTCTAGGAATTCAATC	(SEQ ID NO: 20)
STAT5/6	GTATTTCCCAGAAAAGGAAC	(SEQ ID NO: 21)
IRF-1	AAGCGAAAATGAAATTGACT	(SEQ ID NO: 22)
c-Myb	CAGGCATAACGGTTCCGTAG	(SEQ ID NO: 23)
NFkB	ATATAGGGGAAATTTCCAGC	(SEQ ID NO: 24)
HSINF	CAAAAAAATTTCCAGTCCTT	(SEQ ID NO: 25)
HSIL-6	ATGTTTTCCTGCGTTGCCAG	(SEQ ID NO: 26)
CRENFkB	CTCTGACGTCAGGGGAAATTTCCAGC	(SEQ ID NO: 27)

Please replace paragraph 4 beginning at page 27, line 19 with the amended paragraph/line as follows:

Immature murine dendritic cells were grown from bone marrow cells in GM-CSF conditioned medium according to published protocols. At day 8 to 11 of culture, non-adherent cells were either MHC class II negative, or intermediate (termed immature DC) or high (termed mature DC). FACS(sorting of MHC class II intermediate ~~of~~ or high cells revealed DC-like

morphology. Bacterial DNA or ssDNA containing transcription response elements strongly upregulated CD80 (B7.1) CD86 (B7.2) CD40 and MHC class II molecules on immature DC, as measured by FACS analysis. In addition, i.s. (immune stimulating) DNA containing transcription response element triggered production of high concentrations of IL-12, TNF-(and IL-6. Finally, the i.s. DNA matured/activated DC (derived from sorted MHC class II intermediate cells) expressed professional APC function as assayed in an allogenic "mixed lymphocyte reaction" and in primary T cell cultures stimulated with the superantigen "staphylococcal enterotoxin B" (SEB). It is known that SEB does not require processing but requires professional APC for presentation to naive V(8 T cells. Conversion, as induced by ssDNA, of immature DC into professional APC explains the powerful adjuvant effect of ssDNA containing transcription response elements ~~in~~ in vivo on humoral and T cell response to poorly immunogenic antigens used for vaccination.